SAGE-Hindawi Access to Research
International Journal of Alzheimer's Disease
Volume 2011, Article ID 861072, 11 pages
doi:10.4061/2011/861072

Review Article

Regulation of Cell Survival Mechanisms in Alzheimer's Disease by Glycogen Synthase Kinase-3

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Received 15 January 2011; Accepted 9 March 2011

Academic Editor: Adam Cole

A pivotal role has emerged for glycogen synthase kinase-3 (GSK3) as an important contributor to Alzheimer's disease pathology. Evidence for the involvement of GSK3 in Alzheimer's disease pathology and neuronal loss comes from studies of GSK3 overexpression, GSK3 localization studies, multiple relationships between GSK3 and amyloid β-peptide (Aβ), interactions between GSK3 and the microtubule-associated tau protein, and GSK3-mediated apoptotic cell death. Apoptotic signaling proceeds by either an intrinsic pathway or an extrinsic pathway. GSK3 is well established to promote intrinsic apoptotic signaling induced by many insults, several of which may contribute to neuronal loss in Alzheimer's disease. Particularly important is evidence that GSK3 promotes intrinsic apoptotic signaling induced by Aβ. GSK3 appears to promote intrinsic apoptotic signaling by modulating proteins in the apoptosis signaling pathway and by modulating transcription factors that regulate the expression of proteins involved in apoptosis. Thus, GSK3 appears to contribute to several neuropathological mechanisms in Alzheimer's disease, including apoptosis-mediated neuronal loss.

1. Introduction

Ten years ago we first noted that glycogen synthase kinase-3 (GSK3) appeared to be linked to all of the major pathological mechanisms that had been identified in Alzheimer's disease [1]. Since then, a remarkable amount of new evidence has solidified the central role of GSK3 in Alzheimer's disease neuropathology, as exemplified by this entire issue being devoted to the subject. Among the early identified links between GSK3 and Alzheimer's disease was the discovery that GSK3 promotes the intrinsic apoptotic signaling pathway that may be partly responsible for neuronal loss in Alzheimer's disease [2]. Here we review the multiple cellular pathways influenced by GSK3 that may contribute to changes in cell viability in Alzheimer's disease.

2. Overview of Cell Death in Alzheimer's Disease

Among the known mechanisms that may contribute to loss of neurons in Alzheimer's disease brain, apoptosis has received the most attention. Apoptotic signaling is generally classified as proceeding by either an intrinsic pathway or an extrinsic pathway. Of these, the intrinsic apoptotic signaling pathway has predominated in studies of Alzheimer's disease. Intrinsic apoptotic signaling is most often induced by intracellular damage that leads to mitochondrial release of cytochrome c and the activation of intracellular cysteine proteases called caspases [3], particularly caspase-9 and caspase-3, with a variety of other pro-apoptotic mediators and caspases contributing to the eventual outcome

of apoptosis [4]. Extrinsic apoptotic signaling is initiated by stimulation of plasma membrane death receptors that initiate apoptosis by activation of caspase-8, and subsequent apoptotic signaling can proceed through the mitochondrial pathway or independently of mitochondria by caspase-8-mediated direct activation of caspase-3 [5]. Of these two apoptotic signaling pathways, the intrinsic system has been the focus of the great majority of studies of apoptotic cell death mechanisms in Alzheimer's disease.

3. GSK3 Promotes Intrinsic Apoptotic Signaling

Much evidence indicates that promotion of the intrinsic apoptotic signaling pathway by GSK3 may be particularly important in the apoptosis and neuronal loss that occurs in Alzheimer's disease. This is because GSK3 has been shown to promote apoptosis following a wide range of insults that activate the intrinsic apoptotic signaling pathway [2]. In order to promote intrinsic apoptotic signaling, GSK3 must be active. The major mechanism regulating GSK3 activity is phosphorylation of an N-terminal serine in each of the two paralogs (commonly called isoforms) of GSK3, serine9-GSK3β or serine21-GSK3α. Phosphorylation of these regulatory serines inhibits GSK3, thus signaling activities that reduce GSK3 serine-phosphorylation activate GSK3. The inhibitory serines in GSK3 can be phosphorylated by several different kinases. The most often studied of these is Akt (also called protein kinase B), which itself is activated by multiple receptor-coupled signaling pathways that signal through phosphatidylinositol 3-kinase (PI3K), such as signaling induced by a variety of neurotrophin receptors. Thus, one mechanism by which GSK3 can be activated is by signals that reduce its serine-phosphorylation mediated by Akt or other kinases. A widely used method to study the actions of GSK3β is to express GSK3β with a serine9-to-alanine9 mutation (S9A-GSK3β) to maintain expressed GSK3β fully active. GSK3 also must be phosphorylated on a tyrosine residue for full activity, tyrosine216-GSK3β or tyrosine279-GSK3α. Although the mechanisms regulating tyrosine-phosphorylation of GSK3 are still not well-understood, a number of reports have indicated that GSK3 activity can be increased by signals that increase tyrosine-phosphorylated GSK3.

3.1. Overexpression of GSK3 Is Sufficient to Activate Apoptosis. Overexpression of GSK3 in cells or rodent brains has been shown to induce apoptosis and neuronal death in many reports. The first study of this type showed that that transient overexpression of wild-type GSK3β was sufficient to induce apoptosis in cultured PC12 cells [6]. Furthermore, this report showed that expression of a dominant-negative kinase-dead mutant of GSK3β was sufficient to reduce apoptosis that was induced by inhibition of PI3K, demonstrating that GSK3 is a major mediator of apoptosis in conditions of reduced PI3K activity [6]. Bijur and colleagues [7] extended those findings to show that although relatively low levels of over-expressed GSK3β did not induce apoptosis in human neuroblastoma SH-SY5Y cells, pro-apoptotic signaling was greatly increased by modestly elevated levels of GSK3β, demonstrating that increased GSK3 activity promotes apoptotic signaling induced by a variety of toxic agents [7]. These and other *in vitro* studies demonstrating that increased GSK3 activity can promote activation of the intrinsic apoptotic signaling pathway and that inhibition of GSK3 provides protection from apoptosis have been previously reviewed in detail [1, 2].

The results of *in vitro* studies that showed promotion of intrinsic apoptotic signaling by GSK3 raised the question of whether abnormal increases in GSK3 *in vivo* may contribute to neuronal death in neurodegenerative diseases, such as Alzheimer's disease. One approach to test this that has been productive is to study transgenic mice over-expressing GSK3. Spittaels and colleagues [8] studied transgenic mice over-expressing constitutively active S9A-GSK3β and found hyperphosphorylation of the microtubule-associated protein tau and altered behaviors in sensorimotor tasks in these mice. Mice postnataly over-expressing S9A-GSK3β driven by the thy-1 promoter in neurons exhibited decreased brain volume and cell size, increased neuronal densities, and learning deficits in the Morris water maze [9]. Lucas and colleagues [10] created transgenic mice over-expressing GSK3β in regions specifically relevant to Alzheimer's disease, the hippocampus and neuronal layers I–VI of the cortex. These mice displayed evidence of apoptosis activation, including increased TUNEL staining and caspase-3 activation in the dentate gyrus [10]. Concomitant with increased markers of apoptosis, the GSK3β-over-expressing mice exhibited activated astrocytes and microglia. These mice also displayed deficits in learning in the Morris water maze, but tau filaments were concluded to not be involved in the learning deficits [11]. Further studies of these mice took advantage of the capability of terminating GSK3β overexpression with doxycyclin treatment, which reduced GSK3β levels, reduced tau phosphorylation, increased microtubule polymerization, reduced reactive astrocytosis, restored spatial memory, and decreased levels of active caspase-3 [12]. When the tetracycline-regulated conditional transgenic mice were crossed with mice over-expressing tau carrying a FTDP-17 mutation, GSK3-mediated hyperphosphorylated tau had an increased propensity to form filaments, leading to neurofibrillary tangles (NFTs), and displayed microencephaly at 18 months of age [13]. Expression of constitutively active S9A-GSKβ in the cortex and hippocampus caused hyperphosphorylated tau, neurofibrillary tangles, and morphological changes in neuronal structure [14]. Mice expressing human P301L tau (JNLP3 mice), expressing mutant amyloid precursor protein (Tg2576 mice), and expressing both P301L tau mutation and mutant APP protein (TAPP mice), all displayed increased tyrosine-phosphorylated GSK3α/β in spinal cord and amygdala neurons characterized by granulovacular degenerative granules and neurofibrillary tangles in the JNPL3 and TAPP mice [15]. Avila and colleagues [16] reported that mice over-expressing GSK3β had a 2-fold increase in tau levels and a decrease in dentate gyrus volume, and suggested that increased GSK3β activity, particularly in the dentate gyrus, hinders neurogenesis, thereby promoting the decreased tissue volume. Collectively, these findings in

transgenic mice indicate that GSK3 promotes pathological process associated with Alzheimer's disease, but whether GSK3 promoted decreases in neuronal viability often was not directly investigated due to the difficulty in capturing transient markers of apoptosis in *in vivo* studies.

3.2. Localization of GSK3 in Alzheimer's Disease Brain. Localization studies in postmortem Alzheimer's disease brain have been used to determine if GSK3 is accumulated or activated in areas with prominent neurodegeneration. Pei and colleagues [17] reported increased GSK3α and GSK3β immunoreactivities in plaques and CA1 hippocampal neurons, and co-staining with Congo red indicated that many cells with increased GSK3β immunoreactivity contained hyperphosphorylated tau and neurofibrillary tangles. Subsequently, Pei and colleagues [18] compared non-diseased brains, deemed Stage 0 cases, to Alzheimer's disease-like brains from middle-aged and senescent patients, classified as stages A–C according to the extent of amyloid deposition, and NF I–VI according to the extent of neurofibrillary tangle pathology. They found only moderate active GSK3β staining in normal brains (Stage 0) in neurons of the entorhinal cortex and CA1 and CA2 regions of the hippocampus [18]. Inactive GSK3β staining was pronounced in entorhinal cortical neurons and the hippocampal CA1 region relative to staining for active GSK3. Stage 0/NF I-II brains also had increased inactive GSK3β, relative to active GSK3, immunoreactivity in the entorhinal cortex and hippocampal CA1 region. Stage III/IV brains showed increased tau phosphorylation immunoreactivity (AT8 antibody) and tangle formation in the entorhinal cortex and hippocampal CA1 region, and tangle-containing neurons also had increased active, as well as inactive, GSK3 immunoreactivity, suggesting increases in both GSK3 levels and activity as disease pathology progressed. Stage V/VI brains exhibited AT8 immunoreactivity and tangle inclusions throughout the entorhinal and temporal cortices and the hippocampus. Most inclusion-positive neurons stained intensely for active GSK3, while little or no inactive GSK3 immunoreactivity was recorded in cortical or hippocampal tissues. Collectively, Pei and colleagues [18] clearly defined an Alzheimer's disease progression profile detailing increased tau phosphorylation and increased GSK3β expression and activity in cortical and hippocampal tissues as disease pathology worsened. Ferrer and colleagues [19] also found increased GSK3 immunoreactivity in degenerating neurons characterized by tangle-like inclusions in Stage III and Stage VI postmortem Alzheimer's disease entorhinal cortex and hippocampus. Furthermore, GSK3 colocalized with 40–80% of neurons with hyperphosphorylated tau (PHF-1 antibody), thereby supporting the notion that GSK3 expression and/or activity increases as Alzheimer's disease progresses.

GSK3 immunoreactivity has been reported to be increased at sites of granulovacular degeneration, a pathological characteristic of Alzheimer's disease [15, 19, 20]. Leroy and colleagues [20] reported increased GSK3β and phospho-tyr216-GSK3β immunoreactivity in neuronal cell bodies and dendrites of postmortem human hippocampal tissues. Increases in GSK3 immunoreactivity co-localized specifically with granulovacular degenerative granules, and there were no detectable changes in GSK3 immunoreactivity within neurofibrillary tangles. Ferrer and colleagues [19] also reported increased GSK3 immunoreactivity in granulovacular degenerative bodies located in neuronal cell bodies, and also found increased GSK3 immunoreactivity in glial cells in postmortem human brain tissues.

3.3. Toxicity Associated with Amyloid-β Peptide (Aβ). Substantial evidence has demonstrated that Aβ activates GSK3 by decreasing its inhibitory serine-phosphorylation, which appears to contribute to Aβ-induced increased tau phosphorylation and to Aβ-induced neurotoxicity [21–30]. These studies showing Aβ-induced activation of GSK3 have used a variety of peptides, including A$\beta_{1–40}$, A$\beta_{1–42}$, and the 25–35 peptide fragment, indicating that accumulation of any of these may activate GSK3, although perhaps by utilizing different signaling mechanisms, which remain to be identified. Takashima and colleagues [21–24, 31] first identified a neuroprotective effect of inhibiting GSK3 (at that time also called tau protein kinase-1) against Aβ-induced toxicity. They found that in cultured rat hippocampal neurons Aβ treatment increased GSK3β activity and pretreatment with GSK3β antisense oligonucleotides prevented Aβ-induced cell death and reduced tau phosphorylation. These studies indicated that GSK3 is involved in Aβ-induced tau phosphorylation and neurotoxicity. Subsequent reports also demonstrated that inhibitors of GSK3, such as lithium or SB216763, reduced Aβ-induced tau phosphorylation and cell death in cultured neurons [27, 29, 32]. Inestrosa and colleagues [33] found that treatment with lithium prevented Aβ_{1-42}-induced morphological changes, specifically shrunken soma and affected dendritic and axonal processes, and reduced Aβ-induced decreases in cell viability of primary rat hippocampal neuronal cultures [33]. After injection of Aβ into rat hippocampus, increased GSK3 immunoreactivity was found near Aβ deposits [33]. Treatment with SB216763 or GSK inhibitor VIII also prevented Aβ-induced caspase-3 activation *in vivo*, decreased TUNEL positive neurons, prevented tau-phosphorylation, reduced microglia activation, decreased cytochrome c release from the mitochondria to the cytosol, and improved deficits in the Morris water maze [27, 28]. GSK3 inhibitor VIII or lithium reduced A$\beta_{1–42}$-induced reduction in cell viability and reduced markers of apoptosis [28]. Lithium treatment decreased cortical tau phosphorylation and aggregates, and reduced axonal degeneration [34]. Administration of the GSK3 inhibitor NP12 decreased tau phosphorylation, decreased Aβ deposition, and improved performance in the Morris water maze in amyloid precursor protein (APP) transgenic mice, and reduced neuronal loss in the CA1 region of the hippocampus and the entorhinal cortex [35]. Rockenstein and colleagues [36] also reported neuroprotective effects of inhibiting GSK3 with lithium using APP transgenic mice, with improvements in the Morris water maze task, decreased Aβ immunoreactivity, decreased phospho-tau immunoreactivity, and an increase in MAP2 staining (indicative of increased neuron

density) after treatment with lithium. The role of GSK3 was further examined by crossing mice conditionally expressing a dominant-negative (DN) GSK3β construct with hAPP transgenic mice. These hAPP x DN-GSK3β mice displayed improved performance in the Morris water maze, increased MAP2 immunoreactivity, decreased Aβ immunoreactivity, decreased phospho-tau immunoreactivity, and normal cell morphologies, when compared to hAPP transgenic littermates, suggesting that inhibition of GSK3 can phenotypically rescue hAPP mice [36]. Ma and colleagues [37] showed that antibodies directed against Aβ increased inhibitory serine-phosphorylation of GSK3, which was associated with a decrease in neurotoxicity. Altogether, these and additional reports have firmly established that Aβ activates GSK3 and that reducing GSK3 activity provides protection from Aβ-induced neurotoxicity.

Studies of the mechanism by which Aβ activates GSK3 have indicated the involvement of the PI3K-Akt pathway, which normally maintains inhibitory serine-phosphorylation of GSK3. Aβ treatment was shown to cause time-dependent decreases in PI3K activity and increases in GSK3 activity [22]. Treatment of cultured cells with $A\beta_{1–42}$ reduced Akt phosphorylation, indicative of decreased Akt activity [38, 39], activated GSK3β [38], and activated caspase-3 [25], suggesting that decreased Akt activity contributes to Aβ-induced activation of GSK3, which promotes apoptosis.

In addition to acting downstream of Aβ in its neurotoxic signaling, GSK3 likely also influences the neurotoxicity of Aβ by regulating APP processing and the production of Aβ. Takashima and colleagues [24] found that GSK3β associated with presenilin-1 in postmortem Alzheimer's disease cortical tissues and in COS-7 cells transiently transfected with wild-type presenilin-1, which raised the possibility that GSK3 may regulate Aβ production. This was found in studies that showed reducing GSK3 activity *in vitro* or *in vivo* diminished the production of Aβ [40–42]. The mechanism by which GSK3 promotes Aβ production remains to be determined, but may be related to its phosphorylation and regulation of presenilin-1 [24, 43] or of APP [44].

β-Catenin destabilization has been suggested to be a contributing factor in Aβ-induced GSK3-mediated neurotoxicity. GSK3 promotes the degradation of β-catenin, and nuclear β-catenin levels were decreased in response to acute Aβ treatments, indicating that Aβ-induced activation of GSK3 led to increased degradation of β-catenin [45, 46]. Lucas and colleagues [10] reported decreased nuclear β-catenin levels in GSK3 over-expressing mice. Presenilin-1 (PS1), a GSK3 substrate, can regulate the turnover of β-catenin [47, 48]. Kang and colleagues [48] found that GSK3 co-immunoprecipitated with PS1 but not with mutant M146L or ΔX9 PS1. Overexpression of PS1 also increased the GSK3β-β-catenin association, thereby facilitating GSK3-mediated phosphorylation and subsequent degradation of β-catenin. PS1 mutants were later linked to increased GSK3 activity via decreased PI3K/Akt signaling, thereby promoting decreased inhibitory serine-phosphorylation of GSK3 in primary neuronal cultures [49]. In cultured $PS1^{-/-}$ neurons the activated GSK3 was associated with increased caspase-3 activation [49]. In HEK293 and SK-N-MC cells, Kwok and colleagues [50] transiently over-expressed GSK3$\beta\Delta$exon9+11, which lacks exons 9 and 11 and is characterized by an increased propensity to phosphorylate tau, and found decreased β-catenin levels and signaling. Transient transfection of tau decreased β-catenin levels by 25%, and co-expression of tau and GSK3$\beta\Delta$exon9+11 reversed the GSK3-mediated decrease in β-catenin signaling. Inestrosa and colleagues have reported in detail that activation of Wnt signaling, which inhibits GSK3-mediated phosphorylation and degradation of β-catenin, is neuroprotective against Aβ toxicity [33, 51–53]. Thus, reduced levels of Wnt signalling-associated β-catenin may contribute to GSK3-mediated neurotoxicity induced by Aβ production and promoted by mutations in PS1 in Alzheimer's disease.

3.4. Toxicity Associated with Tau. The microtubule-associated protein tau is one of the most well characterized substrates of GSK3 [54]. Phosphorylation of tau by GSK3 promotes tau dissociation from microtubules, increasing destabilization of microtubules [55]. Conversely, inhibition of GSK3 promotes tau binding to microtubules and assembly of microtubules [56]. As noted above, several studies have reported that the GSK3-mediated increase in tau phosphorylation in Alzheimer's disease may result in part from Aβ-induced activation of GSK3. GSK3-mediated tau phosphorylation in Alzheimer's disease has been suggested to promote tau oligomerization, which can be toxic [57, 58], and aggregation of tau and eventual neurodegeneration [54, 59]. Sahara and colleagues [60] reported that overexpression of tau in SH-SY5Y cells resulted in increased tau phosphorylation and increased caspase-3 activity, suggesting a role in pro-apoptotic signaling and cell death. It is possible that GSK3β-mediated hyperphosphorylation of tau may promote tau-mediated, as well as Aβ-mediated, neurotoxicity.

Transgenic mice have also been used to study the interactions between tau and GSK3. Using protein preparations from the brains and spinal cords of double transgenic mice over-expressing GSK3β and human tau40-1, an isoform of tau containing an additional 29 and 58 amino acid sequence that promotes Alzheimer's disease-like pathologies [61], Spittaels and colleagues [8] found decreased tau binding to microtubules in double transgenic mice, as compared to transgenic mice littermates expressing human tau40-1 alone. The relationship between GSK3 and tau was found to be more than a mere protein-protein interaction, as Kwok and colleagues [50] found interactions between the GSK3β and tau (MAPT) genes associated with increased risk and incidence of Alzheimer's disease. Using senescence-accelerated mice (SAM), Tajes and colleagues [62] showed that inhibition of GSK3 with lithium decreased calpain activation and decreased caspase-3 activity. Primary neuronal cultures treated with the GSK3 inhibitors lithium or SB415286 exhibited decreased neurite disintegration, neuronal shrinkage, and nuclear condensation, further implicating GSK3 in neurodegenerative disease progression [62]. In transgenic mice expressing mutant tau, chronic lithium

treatment reduced tau aggregation [63]. Evidence of tau-related toxicity has been bolstered by studies of tau-knockout mice [64]. Mice conditionally over-expressing GSK3 and lacking tau performed better in the Morris water maze task, as compared to GSK3 over-expressing littermates [64]. Knockout of tau reduced GSK3-mediated shrinkage of the dentate gyrus and reduced reactive microglia, as GSK3-only over-expressing littermates were characterized by increased brain shrinkage and increased reactive microglia compared to control and tau-knockout mice.

In addition to hyperphosphorylation of tau, GSK3 has also been linked to alternate splicing of tau, thereby possibly promoting pro-apoptotic oligomerization and tau-induced cell death [65]. Inclusion of exon 10 likely promotes increased binding of tau and stabilization of microtubules, thereby combating tau aggregate-mediated neurofibrillary tangle formation and neurodegeneration observed in Alzheimer's disease. Hernández and colleagues [65] examined the relationship between GSK3 and alternative splicing of tau and found that in primary mouse cortical neurons treatment with GSK3 inhibitors lithium or AR-A014418 decreased alternative splicing of tau and promoted the increased presence of exon 10 in tau, which promotes microtubule bundling and stabilization, as compared to exon 10-absent tau [65, 66]. Alternative splicing of tau has also been linked to caspase-mediated cleavage and aggregation of tau in Alzheimer's disease [67]. Alternative forms of tau have been linked to increased tau aggregation in other cells and cell systems [68].

In contrast to reports of tau oligomerization contributing to neurotoxicity, a few reports suggest a neuroprotective role for tau. Mouse neuroblastoma cells stably over-expressing tau were less affected by apoptotic stimuli, including staurosporine, camptothecin, and H_2O_2 treatments, and over-expressed tau blocked GSK3 overexpression-mediated increases in cell death, actions that may have resulted from tau binding to GSK3 to block its induction of β-catenin degradation, allowing up-regulated levels of β-catenin, which supports cell survival [69]. Recently, Wang and colleagues [70] found that overexpression of human tau, *in vivo* in transgenic mice and *in vitro* in N2a cells, decreased p53 levels, decreased mitochondrial cytochrome c release, and decreased caspase-9 and caspase-3 activation. Treatment with lithium exaggerated the decrease in p53 expression and increased pro-apoptotic processes [70]. Thus, the connections between tau and GSK3 in affecting neurodegeneration remain to be further clarified and may be complicated by employing overexpression approaches.

3.5. GSK3 Promotes Insults Associated with Alzheimer's Disease. As previously reviewed [2], GSK3 promotes apoptosis induced by many insults that activate the intrinsic apoptotic signaling pathway, some of which may contribute to neuronal loss in Alzheimer's disease. For example, oxidative stress is increased in Alzheimer's disease, as indicated by increased markers of oxidative stress found in postmortem Alzheimer's disease brain [71–73], and has been associated with the loss of neuronal viability, and GSK3 promotes oxidative stress-induced cell death [2]. For example, Schäfer and colleagues [74] found that resistance to oxidative stress was associated with decreased GSK3 activity. Aβ treatment of cells increases oxidative stress [75, 76], as well as activates GSK3, which may contribute to apoptosis. Several reports showed that GSK3 inhibitors reduce toxicity of oxidative stress [77, 78]. Thus, inhibition of GSK3 may be neuroprotective in Alzheimer's disease in part by reducing oxidative stress-induced neurotoxicity.

Neurotrophic factor deficiency has been linked with neuronal loss in Alzheimer's disease. Studies of insulin-like growth factor-I (IGF-1) are particularly interesting because IGF-1 deficiency has been linked to Alzheimer's disease and IGF-1-induced cellular signaling contributes to maintaining inhibition of GSK3 by activating the PI3K-Akt pathway. Additionally, GSK3 inhibition has been linked to increases in IGF-I in the brain [79]. Bolós and colleagues [79] used megalin, an IGF-I receptor interacting protein that is associated with transport of IGF-1, and found that in MDCK cells transiently transfected with or without mini-megalin, a cDNA encoding the two peri-membrane extracellular cysteine-rich domains, the trans-membrane region, and the cytoplasmic region of the megalin gene, treatment with the GSK3 inhibitor NP12 stimulated internalization of IGF-I and cell-surface megalin expression. Moreover, treatment of APP/PS1 transgenic mice with NP12 significantly increased both brain and CSF IGF-I levels. Collectively this data suggests that inhibition of over-active GSK3β that appears to occur in Alzheimer's disease can promote IGF-I expression and counteract Aβ-induced toxicity. Brain-derived neurotrophic factor (BDNF) activation of TrkB receptors is also responsible for activation of the PI3K/Akt pathway and inhibition of GSK3β via Ser9 phosphorylation [80–82]. Decreases in hippocampal and cortical BDNF levels have been reported in Alzheimer's disease [83–85], which could promote an increase in GSK3 activity. Elliott and colleagues [85] showed that in neuronally differentiated P19 mouse embryonic carcinoma cells, BDNF altered tau phosphorylation, and that inhibition of GSK3 with lithium reduced tau phosphorylation. BDNF has also been linked to promotion of anti-apoptotic signaling via the PI3K/Akt pathway. Hetman and colleagues [86] found that trophic factor withdrawal promoted inhibition of the cell survival mediator PI3K and activated the pro-apoptotic GSK3, which was reversed by PI3K activating treatments, such as BDNF, by treatment with a GSK3 inhibitor, or after transient transfection of a kinase-dead GSK3 mutant. Overexpression of wild-type or mutant β-catenin, in which all GSK3β-targeted serines were mutated to alanines, had no effect on GSK3β-mediated neuronal apoptosis [86]. Thus, neurotrophin deficiency in Alzheimer's disease may contribute to abnormally active GSK3 that can promote neurotoxicity.

3.6. Mechanisms by Which GSK3 May Impede Cell Survival from Insults. GSK3 has been reported to promote apoptosis by regulating the actions of proteins involved in apoptosis signaling and by regulating transcription factors known to

regulate the expression of apoptosis modulators. For example, GSK3 has been reported to regulate Bax, a pro-apoptotic Bcl2 family member that is commonly associated with the release of cytochrome c. Under apoptotic conditions, Bax undergoes a conformational change associated with its translocation from the cytosol to the mitochondria where it facilitates cytochrome c release in apoptotic signaling [87–89]. GSK3 can directly phosphorylate Bax on Ser-163, which results in the activation of Bax [90] and inhibition of GSK3 with lithium prevented Bax activation and subsequent cytochrome c release [91]. Another Bcl2 family member, Mcl-1, an anti-apoptotic protein that can be induced after cellular stress to promote cell survival, is phosphorylated on Ser159 by GSK3 to promote Mcl1 degradation, thereby reducing the protective action of Mcl-1 [92, 93]. By these and other actions on the apoptotic signaling pathway, GSK3 can reduce cellular resilience to stress and promote apoptotic signaling.

Several transcription factors that are inhibited by GSK3 normally promote mechanisms that promote cellular survival responses to stresses that are potentially lethal insults [1]. These include heat shock factor protein 1 (HSF-1), cyclic AMP response element-binding protein (CREB), and others, impairments of which are well-documented to increase the susceptibility of cells to toxic insults. HSF-1, for example, promotes the expression of heat shock proteins, chaperones that combat cellular stress. Chu and colleagues [94] reported that GSK3 reduced HSF-1 activity and increased susceptibility to environmental stressors. Xavier and colleagues [95] showed that overexpression of GSK3β repressed HSF-1 transcriptional activity and DNA-binding. CREB, which can support cell survival and is activated by phosphorylation at Ser133, also can be negatively regulated by GSK3 [1, 96]. Activation of CREB has been reported to be impaired in Alzheimer's disease hippocampal tissues [97, 98]. Since GSK3 inhibits CREB activity [1, 96, 99], increased GSK3 activity may contribute to the Alzheimer's disease-induced decrease of phospho-CREB-mediated neuroprotection. Thus, by regulating these and other transcription factors that influence the expression of proteins that modulate cellular responses to stress [1], GSK3 may contribute to setting the threshold for apoptotic signaling, which may be lowered in Alzheimer's disease.

3.7. GSK3 Promotes Decreased Cell Survival in Other Neurodegenerative Diseases. Many components of neurodegenerative processes are common among various neurodegenerative diseases, including apoptosis and mechanisms regulating apoptosis. Thus, it is not surprising that, similarly with Alzheimer's disease, GSK3 has also been linked to neuronal death in other neurological diseases. For example, prion disease shares with Alzheimer's disease accumulations of protein aggregates and neuronal death [100, 101]. Mouse embryonic cortical neurons (E17) treated with varying concentrations of prion protein (PrP) peptide exhibited increased GSK3 activity and increased tau phosphorylation, which was prevented by pretreatment with lithium. GSK3β activation and hyperphosphorylation of tau has also been identified in Lafora Disease, an autosomal recessive form of progressive myoclonus epilepsy that is characterized by dementia and rapid neurological deterioration [102]. Amyotrophic lateral sclerosis has been linked to mutations in superoxide dismutase type 1 (SOD1), and expression of mutant SOD1 in motor neurons increased GSK3 activity and apoptosis, and GSK3 inhibitors provided protection from apoptosis [77]. Thus, there appear to be a variety of disease-associated conditions that can cause abnormal activation of GSK3 that contributes to the neurodegenerative process.

4. GSK3 Impedes Extrinsic Apoptotic Signaling

In contrast to the many studies of intrinsic apoptotic signaling mechanisms in association with loss of cell viability in Alzheimer's disease, few studies have addressed the possibility that death receptor-mediated extrinsic apoptotic signaling is involved in Alzheimer's disease. Plasma membrane death receptors that can initiate apoptosis are members of the tumor necrosis factor (TNF) receptor family that contain conserved intracellular death domains, which includes Fas (CD95/Apo1), TNF-R1 (p55/CD120a), TNF-related apoptosis-inducing ligand receptor-1 (TRAIL-R1/DR4), and TRAIL-R2 (DR5/Apo2/TRICK2/KILLER). Studies in postmortem Alzheimer's disease brain and particularly in Aβ-treated cells *in vitro* have provided some evidence for increased death receptor-induced apoptotic signaling pathway [103–111]. However, the contribution of death receptor-initiated apoptosis in Alzheimer's disease remains to be firmly established.

Although it remains unclear if death receptors contribute to cell loss in Alzheimer's disease, we can surmise that GSK3 is highly unlikely to contribute to this potential neuropathological mechanism. This is because GSK3 impairs death receptor-induced apoptotic signaling, as opposed to its promotion of intrinsic apoptotic signaling [2]. The concept that GSK3 inhibits death receptor-induced apoptosis followed the discovery that GSK3β knockout mice died during embryonic development due to massive hepatocyte apoptosis [112], which demonstrated that GSK3β is an important inhibitor of TNFα-induced apoptosis. This inhibitory effect of GSK3 on extrinsic apoptotic signaling was extended to all other death receptors, as reviewed [2]. The mechanism for this action was found to be due to the presence of GSK3 in a death receptor-associated anti-apoptotic complex that impedes the initiation of apoptotic signaling [113]. Thus, several studies have clearly established that GSK3 is anti-apoptotic in death receptor-mediated signaling.

If death receptor-induced apoptosis does contribute to cell loss in Alzheimer's disease, the anti-apoptotic action of GSK3 in this process could very likely limit the application of inhibitors of GSK3 as therapeutic agents in Alzheimer's disease because they would be able to promote extrinsic apoptotic signaling. This complication was exquisitely demonstrated in a study of the effects of *in vivo* treatment with the GSK3 inhibitor lithium, which demonstrated increased neuronal apoptosis mediated by lithium's promotion of Fas-mediated apoptotic signaling [114]. Whether or not this detrimental action of GSK3 inhibitors would be deleterious

in Alzheimer's disease depends on whether death receptor-induced apoptotic pathways are activated in Alzheimer's disease, a question that remains unresolved.

5. Conclusions

GSK3 has been shown to be associated with the major neuropathological markers of Alzheimer's disease and to be abnormally activated or expressed in Alzheimer's disease brains, particularly in association with neuropathological or degenerative markers. GSK3 is activated by Aβ and promotes both Aβ production and its neurotoxic actions. GSK3 phosphorylates tau and may promote oligomerization of tau and its aggregation, which can contribute to neurotoxicity. Apoptosis may contribute to neuronal loss in Alzheimer's disease, and GSK3 promotes intrinsic apoptotic signaling induced by many insults, some of which may be involved in neurodegeneration in Alzheimer's disease. GSK3 promotes intrinsic apoptotic signaling both by regulating signaling proteins involved in apoptosis and regulating transcription factors that control the expression of proteins that modulate cellular responses to stress. Altogether, much evidence indicates that GSK3 is an integral component of the neurodegenerative processes in Alzheimer's disease.

Acknowledgment

The authors' research was supported by grants from the NIMH (MH092970, MH090236, and MH038752).

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